PATENT COOPERATION TREATY

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MITCHELL, Randall S.
Ridout & Maybee
Suite 2400
One Queen Street East
Toronto, Ontario M5C 3B1
CANADA

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Sent by FAX: 30/03/01

Date of mailing *(day/month/year)*	04.04.01
Applicant's or agent's file reference: 29296-0011	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA 00/00918	04/08/2000	06/08/1999

Applicant

IMI INTERNATIONAL MEDICAL INNOVATIONS INC.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

02/03/2001

2. This date of receipt is: (AMENDED DATE OF RECEIPT)

- [x] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	CHURCHER G Tel. (+49-89) 2399-2545

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Form PCT/IPEA/402 (July 1998) P20452 (30/03/2001)

Express Mail Number

EL634464211 US

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
29296-0011		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00918	04/08/2000	06/08/1999
Applicant		
IMI INTERNATIONAL MEDICAL INNOVATIONS INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   COLOR SPACE ANALYSIS IN BIOCHEMICAL AND IMMUNOLOGICAL ASSAYS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00918

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,7 (partially) 2-6,8-14 (fully)

   Smear test method to analyze liquid and semi-solid body secretions collected from a patient to be diagnosed for evidence of abnormalities such as cancer.

2. Claims: 1,7 (partially) 15-22 (fully)

   Method for analyzing a patient's skin surface for cholesterol.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]CA 00/00918

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 00251 A (BIO METRIC SYSTEMS INC) 11 January 1990 (1990-01-11)	1,7,13
A	page 15, line 32 -page 17, line 9	1,5,12
X	EP 0 893 690 A (UNIVERSITEIT GENT LAB VOOR BRO) 27 January 1999 (1999-01-27)	1,7
A	page 9, line 5 -page 10, line 6	1-6,8,10
X	WO 96 40924 A (CALGENE INC ;MCBRIDE KEVIN (US); PEAR JULIE R (US); PEREZ GRAU LUI) 19 December 1996 (1996-12-19) page 23, line 25 -page 25, line 17 page 45, line 24 -page 46, line 16	1,7
X	WO 90 11526 A (ENZYMATIX LTD) 4 October 1990 (1990-10-04)	1,2,7
A	page 7, line 15 -page 8, line 27 page 2, line 20 - line 31	1,4,5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2001	28. 02. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Tuynman, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 14927 A (KREPINSKY JIRI J ;CHOCIEJ JACEK (CA); KANDEL GABOR P (CA); YEUNG K) 1 June 1995 (1995-06-01)	12-14
A	page 21, line 1 -page 22, line 26; claims 1-10 page 10, line 3 - line 8	1,2,4-6, 8-11
Y	WO 96 08710 A (X RITE INC) 21 March 1996 (1996-03-21)	12-14
A	figures 1,2,5 page 5, line 18 - line 23	1-11
X	GOTO M ET AL: "Chromaticity analysis of Immunostained Tumor Specimens" PATHOLOGY RESEARCH AND PRACTICE, vol. 188, no. 4,5, June 1992 (1992-06), pages 433-437, XP000972430	1,2,7,8, 11
A	abstract page 436; figure 5; table 1	1,4-6
A	EP 0 110 173 A (LIFESCAN INC) 13 June 1984 (1984-06-13) page 7, line 21 -page 8, line 15	1,6, 8-10, 12-14
A	SINCOCK ANDREW: "Computerised laser analysis of breast sections and cervical smears by transnuclear scanning." MEDICAL SCIENCE RESEARCH, vol. 24, no. 3, 1996, pages 165-166, XP000972530 ISSN: 0269-8951 the whole document	1-3,5,6, 12,13
A	GALBRAITH W ET AL: "COLORIMETRY FOR THE STAIN TECHNOLOGIST 4. ANALYSIS OF THE COMPONENTS OF COLOR DIFFERENCE" STAIN TECHNOLOGY, vol. 60, no. 4, 1985, pages 239-246, XP000972409 ISSN: 0038-9153 abstract page 242; table 3	1-3

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 37424 A (LOPUKHIN JURY MIKHAILOVICH ;PARFENOV ALEXANDR SERGEEVICH (RU)) 27 August 1998 (1998-08-27) abstract page 4, line 11 - line 20 page 6, line 23 -page 7, line 7 page 9; example 3 page 11; claims 1,3 figures 1-3	1,15-18, 21,22
A	-& EP 0 987 553 A 22 March 2000 (2000-03-22)	
A	EVELEGH M J ET AL: "Use of skin cholesterol to monitor response to cholesterol-lowering therapy." CLINICAL CHEMISTRY, vol. 45, no. 6 PART 2, June 1999 (1999-06), page A29 XP002160743 51st Annual Meeting of the American Association of Clinical Chemistry;New Orleans, Louisiana, USA; July 25-29, 1999 ISSN: 0009-9147 the whole document	15,16, 18,19
A	US 5 587 295 A (LOPUKHIN JURY M ET AL) 24 December 1996 (1996-12-24) cited in the application column 16 -column 18; example 12 column 19 -column 20; claims 1-8	16-22
A	DE 43 31 010 A (JENOPTIK JENA GMBH) 16 March 1995 (1995-03-16) abstract column 5 -column 6; claims 1-5,7,8	16

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INTERNATIONAL SEARCH REPORT

Inf n on patent family members

International Application No
[redacted]CA 00/00918

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9000251 A	11-01-1990	NONE	
EP 0893690 A	27-01-1999	EP 0892271 A	20-01-1999
WO 9640924 A	19-12-1996	AU 6269196 A	30-12-1996
		CA 2221747 A	19-12-1996
		EP 0835311 A	15-04-1998
		JP 11507233 T	29-06-1999
WO 9011526 A	04-10-1990	NONE	
WO 9514927 A	01-06-1995	AU 687939 B	05-03-1998
		AU 1060095 A	13-06-1995
		CA 2176508 A	01-06-1995
		EP 0731914 A	18-09-1996
		JP 9505405 T	27-05-1997
		US 5416025 A	16-05-1995
		ZA 9409290 A	21-08-1995
WO 9608710 A	21-03-1996	CA 2199868 A	21-03-1996
		CA 2199870 A	21-03-1996
		EP 0781401 A	02-07-1997
		EP 0781404 A	02-07-1997
		WO 9608703 A	21-03-1996
		US 6002488 A	14-12-1999
		US 6031617 A	29-02-2000
EP 0110173 A	13-06-1984	AT 25709 T	15-03-1987
		AU 2087683 A	10-05-1984
		CA 1205731 A	10-06-1986
		DE 3370027 D	09-04-1987
		JP 59097041 A	04-06-1984
WO 9837424 A	27-08-1998	RU 2130189 C	10-05-1999
		AU 5784698 A	09-09-1998
		BR 9807594 A	22-02-2000
		EP 0987553 A	22-03-2000
US 5587295 A	24-12-1996	SU 1675769 A	07-09-1991
		US 5489510 A	06-02-1996
		AT 137336 T	15-05-1996
		AU 615709 B	10-10-1991
		AU 2857889 A	20-07-1989
		BR 8903413 A	15-01-1991
		CA 1335968 A	20-06-1995
		CN 1048925 A	30-01-1991
		CS 8900348 A	14-08-1990
		CS 8905039 A	14-08-1990
		DD 289604 A	02-05-1991
		DE 58909664 D	30-05-1996
		EP 0338189 A	25-10-1989
		FI 890266 A	20-07-1989
		HU 56973 A	28-10-1991
		JP 1289498 A	21-11-1989
		PL 277263 A	04-09-1989
DE 4331010 A	16-03-1995	NONE	

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PATENT COOPERATION TREATY

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PO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 9/830708

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
29296-0011		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00918	04/08/2000	06/08/1999

International Patent Classification (IPC) or national classification and IPC
G01N33/50

Applicant
IMI INTERNATIONAL MEDICAL INNOVATIONS INC.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/03/2001	06.12.2001

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
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Authorized officer

Luis Alves, D

Telephone No. +49 89 2399 8695

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00918

I. Basis of the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-24	as originally filed			

Claims, No.:

1-21	as received on	02/11/2001	with letter of	02/11/2001

Drawings, sheets:

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 15-19, with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. as above relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-6, 8-13, 17-21
	No:	Claims	1, 2, 7, 14-16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-14, 20, 21
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate she t

Section III:

1. The method defined in claim 15 comprises steps performed directly on the human body. Thus, claims 15 and dependent claims 16 to 19 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Section IV:

Claim 1 (partially), 2 to 6 and 8 to 13 (completely) concern smear test methods of analysing liquid and semi-solid body secretions for abnormalities such as cancer and systems for use in said methods.

Claim 1 (partially) and 14 to 21 (completely) concern methods of and systems for analysing a patient's skin for cholesterol.

The single general concept that can be identified as linking said two groups of claims is " a process of analysing a specimen of biological material in a biochemical or immunological test for an analyte, comprising the steps of: a) subjecting the specimen to treatment which develops a colour relating to the amount of analyte in the specimen; b) measuring at least one defined colour characteristic, selected from hue angle, chroma, saturation and lightness of the developed colour; and c) analysing the measurement of said at least one colour characteristic to determine the presence or concentration of said analyte in the specimen".
Said common concept is however not novel (see for example any of WO-A-90/00251, and EP-A-0 893 690, both cited in the International search report).

Therefore, a technical relationship involving one or more of the same corresponding special technical features in the sense of Rule 13.2 PCT is lacking, and the requirement for unity of invention referred to in Rule 13.1 PCT is not fulfilled.

Hence, it is considered that the following separate inventions or groups of inventions are

not so linked as to form a single general inventive concept:

1. Claim 1 (partially), 2 to 6 and 8 to 13 (completely)
2. Claim 1 (partially) and 14 to 21 (completely)

Section V:

Reference is made to the following documents:

D1: WO-A-90/00251
D2: EP-A-0 893 690
D3: WO-A-90/11526
D4: WO-A-95/14927
D6: WO-A-98/37424 & EP-A-0 987 553
D7: WO-A-93/12253
D8: US-A-5 587 295

Documents D1 to D4, D6 and D8 were cited in the International search report.
Document D7 was not cited in the international search report.

1. Invention 1: Claim 1 (partly), claims 2 to 6 and 8 to 14 (completely)

The subject-matter of claims 1, 2 and 7 does not comply with the requirements of Article 33(2) PCT. The subject-matter of claims 3 to 6 and 8 to 13 does not comply with the requirements of Article 33(3) PCT.

D1 discloses an apparatus comprising a reflectance spectrophotometer and an immunoassay making use of said apparatus, in which an analyte in the sample is determined by measuring the chromacity and hue of the developed colour. The analyte consists of carbohydrate markers on chlamydia and as reagents an enzyme- labelled antibody and a substrate/chromogen combination are used.

The sample is applied to a porous substrate, for example nylon (p.7, last paragraph to p.8, second paragraph and p.15, third paragraph to p.17, second paragraph).
Thus, the subject-matter of claim 1 is not novel.
D1 does not specifically describe a kit but it discloses all the components of the kit defined in present claim 12, used together in an assay. Therefore, the subject-matter of claim 12 although novel over D1 (Article 33(2) PCT) cannot be considered to involve an inventive step (Article 33(3) PCT).

D2 concerns detection of mycotoxins using an antibody to the analyte and a colour developing reagent. The sample and reagents are applied onto a microporous membrane. The colour development is correlated to the analyte presence by measuring lightness and chromacity using a colorimeter (see p.9, ln.44 to p.10, ln.1). A portable colorimeter may be used (see p.6, lines 19 to 22). Thus, the subject- matter of claim 1 is not novel over D2 (Article 33(2) PCT).

D3 concerns methods and kits for diagnosing colo-rectal cancer by detecting an analyte in samples such as faeces and other body fluids. The development of colour is measured using for example a portable colorimeter (see abstract and p.7, ln.15 to p.8, ln. 27). The assays are carried out for example in a microtiter plate. Therefore, the subject-matter of claims 1, 2 and 7 is not novel (Article 33(2) PCT). Although not explicitly disclosed that the colour space is measured, implicitly it is, since a colorimeter measures these parameters.

It should be noted that present claim 1 is not restricted to any particular analyte or type of sample, and that the expression "consists essentially of" in present claim 2 is vague and does not restrict the claim to methods in which the measurement is performed directly on the sample, without any pre-treatment steps.

The use of a white substrate and the use of galactose oxidase and Schiff's reagent for detecting carbohydrate markers in the sample is not disclosed in D3. However, the subject-matter of claims 3 to 6 and 8 to 13 does not seem to involve an inventive step for the following reasons: It was already known, for

example from D4, to diagnose colorectal cancer by detecting a disaccharide marker in a colorectal mucus sample by the galactose oxidase test (using galactose oxidase and Schiff's reagent) (see p.5, ln.27 to p.6, ln.15).
D4 also discloses a method of diagnosing colorectal cancer in which Schiff's reagent is used and colour development observed without the use of the enzyme (see claim 3).
The sample is applied to a white non-cellulosic substrate, such as a glass fibre (see p.10, lines 3 to 8).
Therefore, the features in present claims 3 to 6 and 8 to 13 were all well known and their use in the method disclosed in D3 does not seem to involve an inventive step (Article 33(3) PCT).

2. Invention 2: Claim 1 (partly), claims 15 to 21 (completely)

The subject-matter of claims 1 and 14 to 16 does not comply with the requirements of Article 33(2) PCT. The subject-matter of claims 17 to 21 does not comply with the requirements of Article 33(3) PCT.

D6 discloses a method of determining cholesterol in a skin sample by applying cholesterol oxidase and measuring the colour development, after addition of peroxidase and substrate, using for example a portable reflective photometer (see abstract and p.4, lines 45 to 56). Thus, the subject-matter of claims 1 and 14 is not novel (Article 33(2) PCT). Present claim 15 is not restricted to methods in which the step of measuring is performed directly on the patient's skin, without transfer of the sample to a measuring apparatus. Therefore, the subject-matter of claim 15 and dependent claim 16 is not distinguished from the method disclosed in D6.

D7 discloses colorimetric determination of analytes such as cholesterol by adding to the sample an enzyme that oxidates the analyte with formation of hydrogen peroxide (cholesterol oxidase) and observing colour formation after the addition of peroxidase and a dye. The change of hue can be determined either visually or with a spectrophotometer (see p.2, ln.24 to p.4, ln.16 and p.6, ln.25 to p.8, last line, example 1 and Figs. 4 and 5). Thus, the subject-matter of claims

1 and 14 to 16 is not novel (Article 33(2) PCT).

The subject-matter of claim 20 concerns a kit comprising the components already known from any of D6 or D7. Therefore, claim 20 does not seem to involve an inventive step over any of D6 or D7 (Article 33(3) PCT).
D6 discloses carrying out the assay by using a sealing vessel which is fixed on the skin, for example in carrying the assay on the hand of a patient (see abstract, Figs. 1 to 3 and example 1). Therefore, the subject-matter of claim 21 does not seem to involve an inventive step over D6 (Article 33(3) PCT).

The use of compounds as defined in claims 17 to 19 is also already known for the determination of cholesterol and therefore, their use in the methods disclosed in any of D6 or D7 does not seem to involve an inventive step (see D8, claims 1 to 8, column 10, ln.9 and example 12, disclosing the use of a binding agent for cholesterol, such as digitonin, a visualising agent such as peroxidase, a bridging agent such as a copolymer of N-vinylpyrrolidone, and an indicator comprising hydrogen peroxide and N,N-diethyl-p-phenylidene sulfate). Therefore, the use of these reagents in the methods of determining cholesterol disclosed in any of D6 or D7 appears to be merely a normal option known to the skilled person. Therefore, the subject-matter of clams 17 to 19 does not seem to involve an inventive step over any of D6 or D7 when taken in combination with D8 (Article 33(3) PCT).

3. The subject-matter of claims 1 to 14, 20 and 21 appears to be industrially applicable.
For the assessment of the present claims 15 to 19 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to methods for the treatment of the human or animal body by surgery or therapy and diagnostic methods practised on the human or animal body.

Secti n VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1 to D4, D6 and D7 is not mentioned in the description, nor are these documents identified therein.

Section VIII:

1. Claim 7, which defines a substrate, refers back to claim 6. However, in claim 6 there is no mention of a substrate. Therefore, this introduces a lack of clarity into said claim (Article 6 PCT). The same objection applies to claims 8 and 9.

2. The terms "substantially pure white" and "pebbled surface" used in claims 10 and 12, respectively, do not seem to have a well-recognised meaning and therefore render the definition of the subject-matter of said claims unclear (Article 6 PCT).

3. The vague and imprecise statement in the description on page 24, last paragraph, implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

CLAIMS

1. A process for analyzing a specimen of biological material in a biochemical or immunological test for an analyte, comprising the steps of:

subjecting the specimen to treatment which develops a color correlating to the amount of analyte in the specimen;

spectrophotometrically measuring hue angle or chroma of the developed color; and

analyzing the measurement of the hue angle or chroma to determine the presence or concentration of said analyte in the specimen.

2. A process according to claim 1, wherein said specimen of biological material consists essentially of liquid and semi-solid body secretions collected from a human patient to be diagnosed for evidence of abnormalities, said analyte consists essentially of cancer indicating markers in the specimen, and the measured value of the hue angle or chroma is used to classify the specimen as normal or abnormal.

3. The process of claim 2, wherein said secretions are lung mucus, throat mucus, cervical mucus or seminal fluid.

4. The process of claim 2 or claim 3, wherein said specimen is deposited on a generally white substrate and said color developing treatment comprises an enzyme reaction.

5. The process of analyzing a specimen of biological material according to claim 1, wherein:

said specimen of biological material is a colon-contacting semi-solid sample collected from a patient to be diagnosed for evidence of abnormalities;

said analyte consists essentially of markers indicative of the presence of abnormalities;

the step of subjecting the specimen to treatment comprises depositing the specimen on a generally white substrate and developing color from the specimen by enzyme reaction; and

the measured value of the hue angle or chroma is used to classify the sample as normal or abnormal.

6. The process of claim 3, wherein said markers are carbohydrate markers.

7. The process of claim 5 or claim 6, wherein the substrate is non-cellulosic.

8. The process of claim 7, wherein the substrate is glass fibre.

9. The process of claim 5 or 6, wherein the substrate is substantially pure white.

10. The process of any one of claims 5 to 9, said colon-contacting semi-solid sample consists essentially of rectal mucus.

11. A system for analysis of liquid or semi-solid body secretion samples obtained from human patients to diagnose for the presence or absence of abnormalities in the patient, by determination of the hue angle or chroma of a color developed in the sample, said system comprising:

a white, non-cellulosic substrate with a porous "pebbled" surface, for receiving and holding the sample during development;

a source of galactose oxidase, adapted to apply galactose oxidase to the substrate surface for selective enzymatic oxidation of the sample thereon;

a source of Schiff's reagent, adapted to apply Schiff's reagent to the oxidized sample on the substrate for development of analyzable color therein; and

means for presenting the color-developed sample to a portable reflectance spectrophotometer capable of determining the hue angle and the chroma of the color characterizing stained samples on said substrate.

12. A kit for analysis of colon-contacting semi-solid samples obtained from human patients to diagnose for the presence or absence of rectal abnormalities in the patient, comprising;

a generally white, non-cellulosic substrate for receiving the sample;

a source of Schiff's reagent; and

a portable reflectance spectrophotometer capable of determining and reporting the hue angle and the chroma characterizing the color of stained samples on said substrate.

13. A kit according to claim 12 wherein said substrate is glass fibre.

14. A process according to claim 1, wherein said specimen of biological material is the skin surface of a patient and said analyte is skin cholesterol.

15. A process for determining skin cholesterol levels of a patient, comprising:

applying to the patient's skin surface a reagent which selectively binds to skin cholesterol;

causing a color developing chemical reaction with the skin cholesterol-boundary reagent so formed, to form a color complex; and

subjecting the color complex so formed to spectrophotometric analysis to read therefrom the value of the hue angle or chroma characteristic of the skin cholesterol level.

16. A process according to claim 15, wherein said reagent which selectively binds to skin cholesterol is selected from th group consisting of

(i) steroid glycosides, containing as an aglycone a cyclopentaneperhydrophenanthrene fragment of the furostanole or spirostanole

series, and an oligosaccharide fragment including 3 to 10 monosaccharide residues with linear or branched structures,

(ii) triterpene glycosides, containing an aglycone of alpha or beta-amyryl, lupane, hopane, dommarane, linostane or holostane series, and oligosaccharides comprising saccharide residues of branched or linear structure,

(iii) hydrophobic proteins capable of discriminately forming a complex compound with cholesterol,

(iv) protein toxins, capable of discriminately forming complex compounds with cholesterol,

(v) polyene antibiotics, capable of discriminately forming complex compounds with cholesterol, and

(vi) enzymes whose substrate is cholesterol, and which exhibit a high affinity for cholesterol, and

wherein the formation of said colored complex is brought about by treatment of said binding agent on the skin surface first with a visualizing agent and then with an indicating agent.

17. A process according to claim 16, wherein the formation of said color complex is brought about by treatment of said cholesterol binding agent on the skin surface successively with a bridging agent, a visualizing agent and an indicating agent.

18. A process according to claim 16 or claim 17, wherein said cholesterol binding agent is digitonin.

19. A process according to any one of claims 16 to 18, wherein said visualizing agent is peroxidase enzyme and said indicator agent contains hydrogen peroxide, N,N-diethyl-p-phenylidene sulfate, together with appropriate stabilizers.

20. A kit for determination of skin cholesterol levels of a human patient, comprising:

a source of detecting agent, capable of binding to skin cholesterol of the patient to form a bound combination therewith on the skin;

a source of visualizing agent, capable of binding with the detecting agent-binding agent bound combination to form an optically altered complex;

a source of developing agent and means for applying the developing agent to the optically altered complex, to develop color therein; and

means for confining and for presenting said optically altered complex to a portable reflects spectrophotometer to determine therefrom the hue angle or chroma characteristic of the color developed.

21. A kit according to claim 20, wherein said means for confining and presenting the optically altered complex to the spectrophotometer comprises a container in the form of a skin-adherent strip having at least one well passing +therethrough for containment of the reagents in said well in contact against the skin of the patient.

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(54) Title: SPECTROPHOTOMETRIC MEASUREMENT IN COLOR-BASED BIOCHEMICAL AND IMMUNOLOGIGAL ASSAYS

(57) Abstract: A process is provided for analyzing a specimen of biological material in any of a number of biochemical or immunological tests for an analyte which involves subjecting the specimen to treatment which develops a color correlating to the amount of analyte in the specimen. According to the invention at least one defined color characteristic selected from hue angle, chroma, saturation and lightness of the developed color is measured and the results of that measurement analyzed to determine the presence or concentration of the analyte in the specimen. Particular applications are to the detection of cancerous or pre-cancerous abnormalities from the analysis of lung mucus, throat mucus, cervical mucus or seminal fluid.

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(54) Title: COLOR SPACE ANALYSIS IN BIOCHEMICAL AND IMMUNOLOGICAL ASSAYS

(57) Abstract: A process is provided for analyzing a specimen of biological material in any of a number of biochemical or immunological tests for an analyte which involves subjecting the specimen to treatment which develops a color correlating to the amount of analyte in the specimen. According to the invention at least one defined color characteristic selected from hue angle, chroma, saturation and lightness of the developed color is measured and the results of that measurement analyzed to determine the presence or concentration of the analyte in the specimen. Particular applications are to the detection of cancerous or pre-cancerous abnormalities from the analysis of lung mucus, throat mucus, cervical mucus or seminal fluid.

SPECTROPHOTOMETRIC MEASUREMENT IN COLOR-BASED BIOCHEMICAL AND IMMUNOLOGICAL ASSAYS

FIELD OF THE INVENTION

This invention relates to color-based biochemical and immunological assays and tests in which an assay or test sample is subjected to spectrophotometric measurement of color characteristics, in particular, the hue angle and/or chroma. Assays using such measurements have proven useful in producing quantitative or semi-quantitative results in a wide range of medical test and screening procedures and diagnostic methods.

BACKGROUND OF THE INVENTION

As discussed in more detail below, many diagnostic tests depend on the visual examination and appraisal of a color which is developed in a sample of biological material by treatment of the sample with reagents that generate color in positive correlation with the amount of an analytes, i.e. a particular compound to be assayed (e.g. cholesterol) or specific molecular markers present in the sample indicative of a pathological condition, such as cancer.

Tests which require the visual examination and appraisal of the color changes are convenient and often adequate as a preliminary, subjective assessment for the presence of a target compound or a disease marker, but they are essentially non-quantitative.

I have found, surprisingly, that measurement of the hue angle and/or chroma, as well as related color characteristics, by reflective spectrophotometry, affords at least semi-quantitative measurement of the result of assays. From such measurement of one or more defined characteristics of the color of an assay sample derive much valuable information concerning the presence or

absence of progressive diseases such as cancer, as well as their stage of development.

Color-Based Test for Cancer

From U.S. patent 5,162,202, it is known to screen rectal mucous from human patients for detection of colorectal cancer and cancers of the large intestine. The mucous is collected on a membrane filter. A cellulose membrane filter is pre-prepared by impregnation with a solution of the enzyme galactose oxidase in a phosphate buffer, and then lyophilized. At the time of use, the cellulose membrane filter is moistened and then contacted with the membrane filter carrying the mucous sample, for 1-2 hours. Then the mucous bearing membrane filter is washed and reacted with basic fuchsin for 15 minutes, washed and dried. De-colorization of the fuchsin indicates the presence of carbohydrate markers of a cancerous or pre-cancerous condition in the mucous. Such a test is lengthy and tedious to perform, and does not have a high degree of sensitivity, so that it may give false negatives.

An improved rectal mucous test is disclosed in U.S. Patent 5,348,860 Shamsuddin, issued September 20, 1994. In this procedure, the mucous sample is collected and immobilized on a membrane filter, and is treated with galactose oxidase to effect oxidation of any vicinal galactose moieties in the sample to vicinal aldehyde moieties. These are visualized with Schiff's Reagent. This is a more rapid procedure. Samples which test negative by this procedure can be further oxidized with periodic acid and then visualized with Schiff's Reagent, so as to reduce the chances of obtaining false negative results.

A continuing problem with known mucous tests is that the staining results need to be visually examined and appraised. Whilst such examinations are adequate as a preliminary, subjective assessment for the presence of absence of cancer markers, they are qualitative only. They do not give reliable quantitative information about the amounts and concentrations of markers which have been

found, and which could give indications of the state of progression of the cancer condition, if present. Moreover, the medium on which the samples are developed, normally a cellulose membrane, such as filter paper, may itself contain compounds capable of participating in the color development reactions. This can give a "background" which complicates the interpretation of the test results, and reduces its sensitivity. This requires a trained individual skilled in the interpretation of the test result.

Color-Based Assay of Cholesterol Levels

The association of high serum cholesterol levels in patients with propensity to develop atherosclerosis and consequent increased incidence of coronary heart attack, stroke and PVD is firmly established, so that frequent monitoring of patient's cholesterol levels is desirable. More commonly, cholesterol level is determined from extracted blood samples. Many other diagnostic tests are commonly performed also on extracted blood samples, but most of these need only be conducted at longer intervals than cholesterol tests. The invasive nature of the blood collection procedure for cholesterol analysis discourages many patients from undergoing cholesterol monitoring as frequently as is advisable. Accordingly, there is a need for a non-invasive cholesterol test.

It is estimated that the skin contains about 11% of the body's total cholesterol, resulting largely from epidermal steroidogenesis and cholesterol diffusion from blood vessels. It has been postulated that the level of skin cholesterol may more accurately reflect the extent of atherosclerosis than the amount of serum cholesterol.

Nikitin, YP, Gordenko, I.A., Dolgov, A.V. and Filimonova, T.A., "Cholesterol Content in the Skin and its Correlation with Lipid Quotient in the Serum in Normals and in Patients with Ischemic Cardiac Disease", Cardiology 1987 II, No. 10, page 48-51, and others, have demonstrated that there is a close correlation between cholesterol content in the arterial wall and cholesterol

content in the skin of a patient. This suggests a possibility of developing skin tests to determine a patient's cholesterol level. The method described by Nikitin et al., however, involves removing and analyzing skin samples in vitro, a method which is impractical in a clinical setting.

U.S. Patents 5,489,510 and 5,587,295 Lopukhin et al., describe a non-invasive diagnostic test which is performed on the surface of the patient's skin, and which indicates skin cholesterol levels. In the test described in these patents, reagents are provided in the form of affino-enzymatic compounds which are bi-functional in their nature. The bifunctional compound A-B includes a binding agent A which is capable of discriminately forming stable complexes with cholesterol of the skin in order to give the whole bi-functional compound an affinity to cholesterol (for example digitonin); and a visualizing agent B, for example an enzyme such as peroxidase, which permits detection of the bi-functional compounds bound to skin cholesterol. In the practice of this test, a complex of a binding agent A and a visualizing agent B, optionally in combination with a bridging agent C to enhance the sensitivity of the test, i.e. a bi-functional conjugate A-C-B, may be placed on the skin of the palm of the patient. Bridging agent C is suitably a high molecular weight polyfunctional compound such as a polysaccharide or a protein, and serves to space the visualizing agent from the binding agent to minimize steric hindrance of the cholesterol-binding agent reaction. After a suitable incubation period to ensure binding of the complex to the cholesterol of the skin, the area is fully rinsed with clean water to remove unbound reagents. Then the binding area is treated with indicating agent D, to react with visualizing agent B so as to develop color. The greater the cholesterol level, the greater the degree of binding of the bi-functional compound to the skin, and the greater the degree of color development.

A cholesterol test based on the aforementioned patents of Lopukhin et al. has been developed commercially and put into commercial practice. It involves the provision of a kit comprising reagents and a color chart or reader. Most of the reagents are contained in a vial, which the user applies to the test area, on

the palm of the hand, after removing the protective covers. After incubation, the user applies indicating agent and visually assesses the degree of color change which occurs, alongside the color chart or using the reader.

One disadvantage of such a test is the requirement for visual assessment of the color changes. Whilst it is convenient that the test can be carried out by un-skilled personnel, such as the patient, the visual assessment of the resultant color change is subjective and essentially non-quantitative. It can give a valuable indication of cholesterol levels and hence potential problems, but not the type of quantitative measurements which a prescribing physician commonly prefers. The assessment is easily influenced by the nature and color of the background, namely the skin.

It is the general object of the present invention to provide a novel method for determining and measuring the result of a color-based biochemical or immunological assay which does not rely on subjective visual assessment. It is a further and more specific object to provide a method for the quantifiable measurement of the amount of a target compound in a sample of biological material which has been subjected to a biochemical or immunological assay that generates a color in positive correlation with the amount of said target compound.

It is also an object of the present invention to provide a novel test of body secretions, liquid and semi-solid, and a kit for use therein, useful in cancer diagnosis.

It is a further and more specific object of the present invention to provide a novel test of rectal mucous and other secretions, liquid and semi-solid, including stool, and mixtures thereof, and a kit for use therein, which overcomes or at least significantly reduces one or more of the above disadvantages. In the following description, the term "colon-contacting semi-solids" is used to denote mucus, stool and other liquids or semi-solids obtained from the rectum or colon of a

patient, and mixtures thereof, which provide the analyzable material for use in the process of the present invention.

It is also an object of the present invention to provide a novel diagnostic, non-invasive test for cholesterol.

It is a further and more specific object to provide such a test for cholesterol which is capable of producing at least semi-quantitative results.

SUMMARY OF THE INVENTION

With a view to overcoming the disadvantages of prior diagnostic tests based on the requirement for visual assessment of color changes, the present invention in its broadest aspect utilizes certain specific parameters detectable with a spectrophotometer, but not previously used for the present applications. These colorimetric parameters are examined and analyzed, to provide assays and diagnoses of enhanced sensitivity and specificity. The color developed in an assay or test is measured at various wavelengths and the hue angle and/or chroma are measured to provide valuable information concerning the presence or absence of target compounds, disease states or other conditions which are the object of the color-based assay or test. In some cases, the measurement of related color characteristics, such as lightness or saturation, may improve the sensitivity of a test or assay still further.

According to another aspect of the invention, there is provided a process of diagnosing liquid or semi-solid samples of a patient's body secretions for evidence of abnormalities in the tissues or organs from which the secretions emanate, which comprises collecting a liquid or semi-solid body secretion sample from a patient, depositing at least a portion of the sample on a generally white substrate, staining the sample on the substrate and color developing the stained sample, determining a defined color characteristic of the developed color of the

sample by spectrophotometry, and classifying the sample as normal or abnormal according to the value of the defined color characteristic so obtained.

According to a further aspect of the invention, there is provided a system for analysis of liquid or semi-solid body secretion samples obtained from human patients to diagnose for the presence or absence of abnormalities in the patient, by utilization of determination of a defined color characteristic developed in the sample and selected from hue angle, chroma or saturation, and lightness, said system comprising:

a white, non-cellulosic substrate with a porous "pebbled" surface, for receiving and holding the sample during development;

a source of galactose oxidase, adapted to apply galactose oxidase to the substrate surface for selective enzymatic oxidation of the sample thereon;

a source of Schiff's Reagent, adapted to apply Schiff's Reagent to the oxidized sample on the substrate for development of analyzable therein.

and means for presenting the color-developed sample to a portable reflectance spectrophotometer capable of determining and reporting a defined color characteristic selected from hue angle, chroma or saturation, and lightness from stained samples on said substrate.

According to a further, more particular aspect of the invention, there is provided a process of diagnosing rectal colon-contacting semi-solid samples for evidence of abnormalities in the source patient, which comprises collecting a colon-contacting semi-solid sample from a patient, depositing at least a portion of the sample on a generally white substrate, staining the sample on the substrate with galactose oxidase, color developing the stained sample with Schiff's Reagent, determining a defined color characteristic of the developed color of the sample by spectrophotometry, and classifying the sample as normal or abnormal according to the value of the defined color characteristic so obtained.

According to a further aspect of the invention, there is provided a kit for analysis of semi-solid colon-contacting samples obtained from human patients to

diagnose for the presence or absence of rectal abnormalities in the patient, comprising;

a generally white, non-cellulosic substrate for receiving the sample;

a source of galactose oxidase;

a source of Schiff's Reagent;

and a portable reflectance spectrophotometer capable of determining and reporting a defined color characteristic selected from hue angle, chroma or saturation, and lightness from stained samples on said substrate.

According to another aspect of the invention, there is provided a test in which liquid or semi-solid reagents are applied to a patient's skin, to bind to the skin cholesterol, followed by development of color in the reagents, the degree of color development being directly related to the quantity of cholesterol in the skin. Instead of visual assessment, however, the liquid or semi solid reagents in which the color has been developed are analyzed colorimetrically, to determine degree of color development from which cholesterol levels can be at least semi-quantitatively obtained. The chosen colorimetric parameters, such as hue angle or shade, are independent of color density, intensity or lightness (L), and simply measure the color shade. This essentially eliminates the uncertainties introduced from the background color of the skin, so that the test can be conducted on the patient's skin surface. Instrumental colorimetric (spectrophotometric) analysis produces objective numbers which are at least semi-quantitative and indicative of cholesterol levels of the patient.

According to the present invention, in a still further aspect there is provided a process of determining skin cholesterol levels of a patient, which comprises:

applying to a person's skin surface a reagent which selectively binds to skin cholesterol;

causing color developing chemical reaction with the skin cholesterol-bound reagent combination so formed, to form a colored complex;

and subjecting the colored complex so formed to spectrophotometric analysis to read therefrom a pre-defined characteristic of the color of the colored complex.

A still further aspect of the invention is a kit for determination of skin cholesterol levels of a human patient, and comprising:

a source of detecting agent, capable of binding to skin cholesterol of the patient to form a bound combination therewith on the skin;

a source of visualizing agent, capable of reacting with the detecting agent - binding agent bound combination to form an optically altered complex therewith;

a source of developing agent and means for applying the developing agent to the optically altered complex, to develop color therein;

and means for confining and for presenting said optically altered complex to a portable reflectance spectrophotometer to determine therefrom a defined color characteristic selected from hue angle, chroma or saturation.

BRIEF REFERENCE TO THE DRAWINGS

In the description of preferred embodiment of the invention which follows, that portion relating to the aforementioned aspect of the invention relating to a process and a kit for determining skin cholesterol levels of a patient, reference is made to the drawing figures, in which:

Figure 1 is a diagrammatic illustration of a test strip for use in determining the skin cholesterol levels of a patient according to the present invention;

Figure 2 is an illustration of a spectrophotometer reader in use in measuring skin cholesterol levels according to the present invention, and in its open position;

Figure 3 is a view similar to Fig. 2, but with the spectrophotometer in the closed position;

Figure 4 is a detail view of the lower portion or shoe of the spectrophotometer shown in Figure 2 and Figure 3.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As evident from the foregoing discussion, applicant's basic invention of using colorimetric measurements, particularly of chroma and/or hue angle for measurement of the results of color-based assays and tests finds application in a number of aspects and embodiments. Two aspects of the invention and the preferred embodiments thereof will therefore be described further below, separately under individual headings.

Suitable spectrophotometers for use in all aspects of the present invention are portable, reflectance-based, and give accurate measurements of color characteristics such as hue angles, lightness and chroma or saturation, when the incident light of the spectrophotometer is reflected back from the stained sample to the instrument's receiver. They are commercially available. A specific example of a suitable such instrument is that marketed by X-Rite, Grand Falls, Michigan, U.S.A as "Model CA22 Spectrophotometer. It is supplied with appropriate software so that it can be connected to a computer to give an accurate read-out of the hue angle of the stained sample under test. The spectrophotometer receives reflectances over the approximate wavelength 400-700 nm, i.e. over most of the visible light spectrum, suitably over about 20 nm intervals.

It is known that color may be defined and expressed in terms of hue angle. The concept of "hue angle" is defined and discussed in standard textbooks such as "Principles of Color Technology," by Fred W. Billmeyer and Max Saltzman, published by John Wiley and Sons (see particularly Chapters 1 and 2), incorporated herein by reference. "Hue" is the color or shade of a specimen independent of its brightness or intensity, and "hue angle" of a color or shade is the definition of its reflectance wavelength by angular position with

reference to a standard three dimensional ellipsoidal continuum plot of the entire spectrum of visible light. The visible light (color) continuum is represented on an angular scale from 0 to 360°, and the angular values as read by the reflectance spectrophotometer are transformed into linearized form to give the transformed "hue angle" used in the process of the present invention.

(i) Test for Cancer

It has been surprisingly found, in accordance with the invention, that the presence of a whole range of pathologies, included bowel pathologies, lung pathologies, cervical pathologies and others,can be determined by determining the hue angle or other defined color characteristic mentioned above, of the color developed from a liquid or semi-solid body secretion from the appropriate tissue or body organ. Reaction and spectrophotometric analysis of rectal mucus, will serve to diagnose colon cancer. Thus, individuals with rectal cancer give rectal mucus samples which after staining and color developing as described, have higher hue angle numbers than those with normal bowels. Thus the hue angle or other defined color characteristic of the stained sample can be used to differentiate individuals with cancerous lesions of the bowel from those without such lesions. More specifically, samples from cancerous lesions have been found to give hue angles generally in the range 375-425°, the top quartile of measurement from clinical samples. Further, because the test result is interpreted by the portable spectrophotometer, there is no requirement that the test results be produced by a skilled, trained individual.

In a similar manner, lung cancer and lung pre-cancerous conditions can be diagnosed by subjecting lung mucus or sputum to similar color development and spectrophotometric analysis. Cervical cancer and pre-cancer can be diagnosed by such procedures applied to cervical mucus. Seminal secretions such as semen can be similarly analyzed, for cancer of the reproductory organs such as testicular cancer. Mucus of the throat can be similarly analyzed for detection and diagnosis of throat cancer. Mucus of the throat and the lungs can

be obtained by known procedures, such as bronchoscopy or bronchio-alveolar washings. Breast nipple aspirate is a body fluid which can similarly be analyzed by the method of the invention, in testing for breast cancer.

A significant aspect of the preferred embodiments of the present invention is the use of a porous glass fiber membrane on which the sample is oxidized and color developed. Such as glass fiber material is essentially free from stain-producing residues, so that it presents no residues which will undergo enzymatic oxidation so as to participate in the subsequent color developing reaction. Accordingly, background color development likely to confuse or interfere with the diagnostic tests, is effectively eliminated. Moreover, the membrane is essentially pure white in color, further reducing background "noise" against which the results are read.

A further characteristic of the glass fiber membrane used in this aspect of the invention is its surface porosity, which allows additional spreading of the mucus sample thereon so as to expose additional carbohydrate markers in the sample to participate in the oxidation and color development reactions, with consequent improved sensitivity of test method.

A specific, preferred example of a glass fiber porous membrane for use in the present invention is that available commercially from Whatman Inc, Laboratory Division under the designation "Whatman 934-AH Glass Microfiber Filter", a borosilicate glass filter medium having high loading capacity and high retention efficiency at high flow rates. It is recommended for use in cell harvesting and liquid scintillation counting techniques. This is, however, exemplary only, and other, substantially pure white glass fibre, carbohydrate free substrates of surface porosity suitable to effect surface spreading of the colon-contacting semi-solid sample can also be used.

In a specific procedure using a preferred aspect of the present invention, as applied to colon cancer detection and using an enzymatic oxidation reaction

for color development, first the sample to be tested is obtained from the patient. Lubricant is supplied to the gloved finger of the operator. The finger is inserted into the rectum of the patient and rotated 360° to obtain a representative sample of colon-contacting semi-solid such as rectal mucus. The finger is removed from the rectum and the sample is smeared onto the surface of the white membrane filter described above, mounted on a rectal mucus test card with appropriate covering, protection and identification, and the card is analyzed.

For analysis, the backing is removed from the rectal mucus test card, and 50µL of standard galactose oxidase solution is added to the test card. Incubation proceeds, in the standard way, for 10 minutes. Then the card is dipped into double distilled water for 30 seconds and then 1 mL of Schiff's Reagent is applied, for 3 minutes. The color is then developed by transferring the card through four water rinses for 10 minutes each rinse. The card is allowed to dry, and the score is determined by reading the hue angle with the portable spectrophotometer, of the type previously described. A score of hue angle below a certain predetermined value (350 in the case of rectal mucus samples) indicate a normal, healthy tissue origin. Scores over a certain predetermined value (370 in the case of rectal mucus) indicate a cancerous tissue origin.

Samples giving intermediate values may be subjected to universal oxidation to assist in final diagnosis. Based on the knowledge that, in any sample, only a proportion of the vicinal hydroxyl groups on the carbohydrate marker will have enzymatically oxidized to develop color, one can subsequently oxidize all of the remaining such groups with a powerful oxidizing agent such as periodate, and then redevelop the color and test for it. If this makes a large difference in comparison with the original result, it indicates that the sample should be classified as if it had given a higher result initially. If it makes only a minor difference, or no significant difference, the sample can be more safely grouped with those giving the lower results.

From the values of hue angle simply read out of the spectrophotometer in this way, the operator can, without any subjective interpretation, determine whether the sample originates with a patient having healthy, normal colon or cancerous colon, with greatly reduced chances of false positive or false negative readings, as compared with prior diagnostic methods. The same standard collection, staining, incubation and color development steps are taken, and the same standard reagents are used, so that the new procedure according to the invention can be adopted by established diagnostic laboratories with minimum disruption and economic expenditure.

Essentially similar procedures are adopted in respect of other mucus samples from other body organs and tissues. Enzymatic oxidation with galactose oxidase, followed by reaction with Schiff's Reagent, is preferred as the color developing reaction procedure for subsequent color analysis according to the invention. However, the invention is not limited thereto. Any procedure which will result in selective reaction resulting in color development characteristic of the cancer development thereof, can be employed. For example, direct reaction of the sample with Schiff's Reagent, without an enzymatic reaction step, can be used for color development. In all cases the spectrophotometric analysis of the developed color to determine an objective parameter of the shade or hue of the color, e.g. hue angle constitutes the use of the present invention, such color parameters having been found to correlate with the presence and the extent of progression of various cancers.

(ii) Non-Invasive Cholesterol Test

The preferred process of this aspect of the invention employs liquid or semi-solid biochemical reagents, develops color therein determinative of the patient's skin cholesterol content, and subjects the color so developed to spectrophotometric analysis. According to the invention, the precise nature and identity or shade of the color so developed, as characterized for example by its hue angle, correlates with the amount of bound complex formed and hence with

the cholesterol content of the skin. This measurement of color characteristic is objective and at least semi-quantitative. Accordingly, it is read independently of background skin color and is not influenced thereby, to any significant extent.

It is usually convenient to add all the reagents, in the appropriate order, to the patient's skin surface, allow color development on the skin surface, and then examine the color-developed complex spectrophotometrically, while it remains on the skin. The entire test can be conducted in under five minutes. The area of skin chosen for the test should be one which is essentially free from sebaceous glands, since such glands contribute cholesterol-containing sebum which would interfere with the results. The sole of the foot and the palm of the hand are suitable such skin areas, with the palm of the hand being the most convenient for use in the present test.

The kit includes a means for confining and presenting the color-developed complex for analysis by a portable spectrophotometer. Suitably this is a container in the form of a skin-adherent strip, with one or more wells passing therethrough, so that reagents contained in the wells can contact the patient's skin. Container design will largely be dictated by the physical characteristics of the spectrophotometer. Instead of a container for the reagents, an inert thixotropic agent can be included with the reagents, to limit their spread across the skin surface and to prevent the mixing of test reagents with control reagents applied to adjacent skin locations.

As with other color-based tests and assays to which the present invention is applicable, it is a feature of the non-invasive cholesterol test according to this aspect of the invention that the hue angle of the colored complex bound to the skin cholesterol correlates with skin cholesterol content.

Suitable chemical reagents for use according to this aspect of the intention are generally those described in the aforementioned Lopukhin et al. patents, the disclosures of which are incorporated herein by reference. Their

precise choice is not an essential or limiting feature of the present invention, provided that their use in combination with one another results in the chemical development of color as a result of binding to skin cholesterol. The term "binding" is used herein in its broad sense of chemical reaction to cause attachment of one chemical entity to another, as well as specific affinity-type "gripping" interaction often encountered in biochemical systems.

Thus, the binding agent A is selected from a group of substances capable of discriminately forming stable complexes with free cholesterol of the skin in order to give the whole bi-functional compound in which it is involved an affinity to cholesterol. It can form a stable complex by direct reaction with cholesterol, before or after it is chemically attached to the visualizing agent B directly or through the bridging agent C.

Representative classes of compounds suitable as cholesterol binding agents A include:

steroid glycosides, containing as an aglycone a cyclopentaneperhydrophenanthrene fragment of the furostanole or spirostanole series, and an oligosaccharide fragment including 3 to 10 monosaccharide residues with linear or branched structures (Hinta P.H. "Structure and biological activity of steroid glycosides of spirostan and furostan series", Hishinev, Stinza, 1987, pg. 142) specific preferred examples of which are funcosides C, D, E, F, G and I, dioscin, rocosides C, D and E, lanotigonine, digitonin and tomatine;

triterpene glycosides, containing an aglycone of alpha or beta-amyryl, lupane, hopane, dommarane, linostane or holostane series, and oligosaccharides comprising saccharide residues of branched or linear structure (Deknnosidze G.E., Chirva V.Y., Sergienko T.V., Uvarova N.L. "Study on Triterpene Glycosides", Tbilisi, Mesniereba, 1982;

hydrophobic proteins capable of discriminately forming a complex compound with cholesterol (Himov A.N., Titova G.V., Kozhevnikov H.A., Biochemistry, 1982, Vol. 47, No. 2, pg. 226-232); Himov A.N., Hozhevnikov H.A., Klyueva N.N. et al. Voprosy Meal., Hhimii, 1984, Vol. 30, No. 3, p. 86-90; Titova G.V., Hilyueva N.N., Hozhevnikov H.A., et al. Biochemistry, 1980, Vol. 45, No. 1, pg 51-55);

protein toxins, capable of discriminately forming complex compounds with cholesterol. They are obtained from bacteria, marine microorganisms, insects of snakes (Dalin M.V., Fish N.G. "Protein Toxins or Microorganisms", Moscow, Medicine, 1980); or

polyens antibiotics, capable of discriminately forming complex compounds with cholesterol (I.J. Katzenstein, A.M. Spielvogel, A.W. Norman, J. Antibiot., 27, 12, 1974, pg. 943-951; Jong Shan Shyng, Wang Hsi-Hua, Clin. J. Microbiol., 1976, 9, (1-2), pg. 19-30; Readio Josphine D. et al. Biochim. Biophys. Acta, 1982, 685 (2), pg 219-24); or

high affinity enzymes, whose substrate is cholesterol, and which have a high affinity to it. All of the above-mentioned publications are incorporated herein by reference.

The most preferred choice for cholesterol binding agent A is digitonin.

Visualizing agent B is commonly an enzyme, since enzyme/substrate reactions resulting in a color change are particularly useful. Specific examples of suitable such enzymes include acetyl choline sterase, tyrosinase, glucose-6-phosphate dehydrogenase, glucose oxidase, glucoamylase, beta-D-galactosidase, peroxidase, alkaline or acid phosphatase, alpha-chymotrypsin, and pyrophosphatase. Peroxidase is a preferred choice e.g. horseradish peroxidase (HRP).

The use of a bridging agent C enhances the technical performance of the method, and facilitates the production of the resulting, desirable A-C-B complex from which color can be developed, whilst preserving the functional activity of agents A and B. The most preferred A-C-B complexes are those which use steroid glycosides, which contain as an aglycone, a cyclopentaneperhydrophenanthrene fragment from the furostanol or spirostanol series and oligosaccharide fragments including two to 10 monosaccharides residues with linear or branched structures such as digitonin as the cholesterol affinity binding agent A. It is particularly desirable to use a bridging agent C when digitonin is chosen as the cholesterol binding agent A and HRP is chosen as the visualizing agent, since HRP is a relatively large molecule which, if bound directly to digitonin, might sterically hinder the reaction of digitonin with skin cholesterol. As bridging agent C for such purposes, it is preferred to use high molecular weight polyfunctional compounds. Their use allows a wide range of control over the proportion of agents A and C in the final complex. Such high molecular weight polyfunctional bridging agents C may be various polysaccharides, proteins or synthetic polymers, i.e. any suitable high molecular weight compound containing primary amine, carboxyl, hydroxyl, aldehyde, haloidanhydride, mixed anhydride, iminoester, azide, hydroxide, maleimide, isocyanate, or epoxide functional groups. Copolymers of acrylic acid or maleic acid or maleic anhydride and N-vinylpyrrolidone are the most preferred high molecular weight polyfunctional bridging agents C. Asymmetric low molecular weight bi-functional compounds such as bromocyan, trichlorotriazine or 2-amino-4,6-dichloro-3-triazine can also be used.

Indicating agent D typically contains a substrate of the enzyme employed as the visualizing agent B, and additional compounds needed to make the reaction between the enzyme and its substrate visible. A specific example of such an indicator agent D, for use with peroxidase enzyme as visualizing agent B, is an agent containing hydrogen peroxide, N,N-diethyl-p-phenylidene sulfate, together with appropriate stabilizers. Indicator agent D is selected in combination

with visualizing agent B, from the range of compounds known in the art which will generate color developing reactions in combination with the chosen enzyme.

For conducting the test according to the invention, a kit is provided. The kit includes the required reagents in appropriately sealed packages such as vials or bottles equipped with droppers, a container or other confining means in which the color developing reactions can be conducted, on the patient's skin, whilst preventing spread of the reagents over too wide an area, and from which developed color can be presented to a means for determining and reporting a defined colored characteristic such as hue angle, e.g. a portable reflectance spectrometer, for examination and measurement. The container is suitably an adhesive strip provided with one or more cutout wells, initially provided with a protective backing to protect the adhesive. Preferably the container has at least two or three wells, so that control experiments can be conducted alongside test experiments. To facilitate the correct conducting of the test experiments and controlled experiments, the wells are conveniently made visually distinct from one another, e.g. of different shapes.

Figure 1 of the accompanying drawings illustrates such a container for use in the present invention, in the form of a test strip 10, of rectangular shape. The strip comprises a foam pad 10, with a layer 12 of skin compatible adhesive temporarily protected by a peelable release sheet 14. A first central well 16 for test purposes, of circular cross-section, extend through the foam body of the strip, and through the adhesive layer 12. A second well 18, for positive control purposes, of diamond cross-section, and a third well 20, for negative control purposes, of square cross-section, are similarly provided in the foam body of the strip 10, flanking the central well 16. The different visual shapes of the wells assist the operator in conducting the tests, by aiding the correct choice of well for its respective purpose.

The test is conducted preferably on the skin of the palm of the patient's hand. The container for the reagents, in the form of an adhesive strip, is

temporarily adhered to the skin so that the open bottom of the wells contact the skin. Reagents are dropped into the wells, the color is developed in the wells, and then the color is read by the spectrophotometer without removing the strip from the skin. For this purpose, a specially designed spectrophotometer, constituting another feature of the invention, is used. The spectrophotometer transmits readings to a computer for analysis. The spectrophotometer is designed to ensure proper alignment over the test cell.

Accordingly, this aspect of the invention provides a spectrophotometer adapted to transmit signals from reading color reflectance of a test sample to a computer, the spectrophotometer having a body, a light emitting means in said body, an apertured lower portion of said body through which light emitting means may be directed to shine light, and a recess in the lower surface of said lower portion, adapted to fit over the welled test strip applied to a patient's skin surface to provide precise registry of the spectrophotometer and the test sample in a well of said welled test strip. Preferably, the lower portion of the spectrophotometer is hinged to the body, so that it can be conveniently fitted into proper registry with the test strip while in the open position, and then closed to the body of the spectrophotometer for taking measurements. Preferably also, the hinged lower portion and the body of the spectrophotometer are provided with electrical contacts to act as a switch, to turn on the light of the spectrophotometer when the lower portion is closed to the body of the spectrophotometer.

Figures 2, 3 and 4 of the accompanying drawings diagrammatically illustrate the spectrophotometer. It has a body 22, with electrical connection (not shown) to an appropriately programmed computer for analysis of the results read by the reader. A lower portion 24 is provided, hingedly connected at 26 to the body 22. The lower portion 24 is apertured at 28. A groove 30 extend across the width of the under-surface of the lower portion 24, projecting upwardly from the lower most surface. The groove is closed at one side of the lower portion. The width of the groove 30 is designed to be a precise, tight fit over the test strip 10 shown in Figure 4. An end of test strip 10 is brought into registry with the

closed end of the groove, when a reading is to be taken, and this, combined with the fit of the test strip 10 within the width of groove 30, provides precise registry of the test well 16 with the beam of light to be emitted from the body 22 of the reader, through the aperture 28. The spectrophotometer contains appropriate detector means for picking up reflectance signals from the sample in the well 16, and transmitting them for analysis and read out by the computer. The body 22 and the lower portion 24 are provided with respective electrical contacts 32, 34 which close as a switch when the lower portion 24 is closed to the body 22, thereby switching on the light for taking a measurement.

A specific test procedure will now be described, by way of specific, but non-limiting, example of the practice of the diagnostic test of the present invention.

The kit components include a dropper bottle containing detector solution (digitonin horseradish peroxidase cojugate in an aqueous buffered solution with less than 0.01% of bromonitrodioxane and methylisothiazolone as preservatives, 1.5mL), with a distinctive colored cap (green); a dropper bottle of more highly concentrated detector reagent solution, similarly buffered and preserved with less than 0.01% bromonitrodioxane and methylisothiazolone containing to act as a positive control (1.5ml), and with a distinctive cap (red); an indicator dropper bottle containing a solution of reagents (4.0 mL of 3,3',5,5'-tetramethylbenzidine, TMD, - hydrogen peroxide solution with 5% N,N-dimethylformamide as preservative) to react with the detector and PC reagents that are bound to skin cholesterol, to produce a blue-green color, being equipped with a distinctive cap (blue); foam pads as illustrated in Figure 1, to which the reagents may be added, alcohol swabs and appropriate directions for use. The chemical reagents are storage stable in a refrigerator at 2-8°C for extended periods. The system also includes a spectrophotometerer as illustrated in Figures 2 and 3, connected to an appropriately programmed computer, and a calibration plaque for use with the spectrophotometer. The kit as supplied does not include a spectrophotometer,

except perhaps when initially sold, the same spectrophotometer being re-used with subsequent "refill" kits.

Initially, the spectrophotometer is appropriately calibrated by inserting the calibration plaque into the jaws thereof, and closing it to illuminate the calibration plaque to feed calibration readings back to the spectrophotometer and computer. A signal is eventually received that calibration has been successfully completed.

The patient's hand is washed and rinsed thoroughly with soap and water and well dried. An outside portion of the palm of the patient's hand is then thoroughly cleaned with an alcohol swab, with sufficient scrub pressure to ensure thorough cleanliness. After allowing the hand to dry, release sheet 14 is removed from a test strip 10, which is then adhesively applied to the cleaned skin area of the patient's palm. The patient inverts the hand on a paper towel placed on a tabletop, and firmly pressed down to ensure that the pad is properly adhered to the palm.

Next, the reagents are added to the respective reagent wells. One drop (42 μl) of detector solution is added to round test well 16, one drop of positive control solution is added to diamond-shaped cross-section well 18, but no liquid is added to the third, square well 20 at this point. Incubation of the added solutions is allowed to proceed for 1 minute, whilst the patient holds the test hand stationery. The patient then inverts the palm, and presses the foam pad on paper towel to remove liquid from the wells. Visual inspection is undertaken to ensure that the pad and the test wells are completely dry. Then the patient rests the hand on a flat surface with the palm facing upwards.

Next, one drop of indicator is added to all three wells, including the square well previously unused, and reaction is allowed to proceed for 2 minutes, whilst the patient holds the hand stationery. Immediately afterwards, the reader 22 is put into position over test well 16, closed and a reading is taken of the color

developed in the test well, transmitted to the spectrophotometer and analyzed by the computer, to give a read out of a value of hue angle.

Visual inspection of the positive control well 18 and the negative control well 20 is undertaken. If the liquid from the negative control well is colorless, and the liquid from the positive control well is colored, the test is valid. No quantitative measurements are taken of the color developed in the positive control. This is color developed from a very high solution concentration of reagent, to give a color with even very small amounts of cholesterol on the skin, and is simply indicative of the potency of the reagents, etc., for control purposes.

The residual liquid from the test strip is discarded, and the test strip is removed from the palm of the hand, followed by cleaning of the palm of the hand with an alcohol swab.

The spectrophotometer used in the present preferably measures absorbance of reflected light from the test sample, and converts it through a suitable algorithm to a value of hue angle. In the specific case of the development of color from horseradish peroxidase - TMD reaction described above, absorption at 450 nm, A_{450nm}, is a suitable measurement. Optical density of the absorbance at 450 nm has been experimentally determined to relate to hue angle through the relationship:

$$h°(\text{degrees}) = 490.45 \times A_{450\ nm} + 57.124$$

This relationship is determined by measuring optical density from a series of serially diluted reaction samples at 450 nm, measuring the hue angle of the same samples, and plotting the results on a regression curve to determine the relationship. Similar relationships can be worked out experimentally in the same way for other chosen color developing tests using different enzyme - substrate pairs which develop different colors, to allow optical density recordings to be transformed into hue angle determinations.

Although specific diagnostic tests and kits for carrying them out have been described above, one of ordinary skill will appreciate that a large number of color-based assays and analyses could take advantage of the novel measurements based on hue angle or chroma disclosed herein, for example:

(i) quantification of the outcome of solid phase immunoassays such as dot blots, immunochromatographic tests and flow-through tests (sample added to a membrane with a wicking device behind it, the target analyte being then trapped and identified by the addition of a labeled detector, such as antibody tagged with enzyme or gold;

(ii) quantification of microparticle-based assays using colored beads;

(iii) as an alternative to densitometry in the analysis of stained gels;

(iv) for the quantification of Western blot analysis.

The method of the invention affords a simple approach to quantifying such results and thus supplementing information gathered from the assay to permit the application of more sophisticated techniques of statistical analysis to the test results.

Accordingly, variations to the invention are not to be regarded as a departure from the spirit and scope thereof, and all such modifications as would be obvious to one skilled in the art are intended to be included in the scope of the following claims.

CLAIMS

1. A process of analyzing a specimen of biological material in a biochemical or immunological test for an analyte, comprising the steps of:

subjecting the specimen to treatment which develops a color correlating to the amount of analyte in the specimen;

measuring at least one defined color characteristic, selected from hue angle, chroma, saturation and lightness of the developed color; and

analyzing the measurement of said at least one color characteristic to determine the presence or concentration of said analyte in the specimen.

2. A process according to claim 1, wherein said specimen of biological material comprises liquid and semi-solid body secretions collected from a patient to be diagnosed for evidence of abnormalities,

said analyte consists essentially of cancer indicating markers in the specimen, and

the measurement of said at least one color characteristic is used to classify the specimen as normal or abnormal according to the value of the color characteristic so obtained.

3. The process of claim 2, wherein the sample is lung mucus, throat mucus, cervical mucus or seminal fluid.

4. The process of claim 2 or claim 3, wherein the sample collected from a human patient is deposited on a generally white substrate, and the process includes developing color from said sample by enzyme reaction, determining at least one defined color characteristic selected from hue angle, chroma or saturation, and lightness, of the color so developed, and classifying the sample as normal or abnormal according to the defined characteristic of the color so developed.

5. The process according to claim 1, wherein said specimen of biological material comprises colon-contacting semi-solid samples collected from a patient to be diagnosed for evidence of abnormalities

said analyte consists essentially of carbohydrate markers indicative of abnormalities,

the step of subjecting the specimen to treatment comprises depositing the specimen on a generally white substrate, staining the sample on the substrate with galactose oxidase and color developing the stained sample with Shifts reagent, and

the measurement of said at least one color characteristic is used to classify the specimen as normal or abnormal according to the value of the color characteristics so obtained.

6. The process of analyzing a specimen of biological material according to claim 1, wherein said specimen of biological material comprises a colon-contacting semi-solid sample collected from a patient to be diagnosed for evidence of abnormalities,

said analyte consists essentially of markers indicative of the presence of abnormalities,

the step of subjecting the specimen to treatment comprises depositing the specimen on a generally white substrate and developing color from the specimen by enzyme reaction; and

the measurement of said at least one defined color characteristic is used to classify the sample as normal or abnormal according to the defined characteristic of the color so developed.

7. The process of any previous claim, wherein the defined color characteristic is the hue angle, and the hue angle is determined spectrophotometrically.

8. The process of any one of claims 2 to 6, wherein the substrate is non-cellulosic.

9. The process of any one of claims 2 to 6, wherein the substrate is glass fibre.

10. The process of any one of claims 2 to 6, wherein the substrate is substantially pure white.

11. The process of any one of claims 5 to 10, wherein the sample is predominantly a rectal mucus sample.

12. A system for analysis of liquid or semi-solid body secretion samples obtained from human patients to diagnose for the presence or absence of abnormalities in the patient, by utilization of determination of a defined color characteristic developed in the sample and selected from hue angle, chroma or saturation, and lightness, said system comprising: a white, non-cellulosic substrate with a porous "pebbled" surface, for receiving and holding the sample during development;

a source of galactose oxidase, adapted to apply galactose oxidase to the substrate surface for selective enzymatic oxidation of the sample thereon;

a source of Schiff's Reagent, adapted to apply Schiff's Reagent to the oxidized sample on the substrate for development of analyzable therein.

and means for presenting the color-developed sample to a portable reflectance spectrophotometer capable of determining and reporting a defined color characteristic selected from hue angle, chroma or saturation, and lightness from stained samples on said substrate.

13. A kit for analysis of colon-contacting semi-solid samples obtained from human patients to diagnose for the presence or absence of rectal abnormalities in the patient, comprising;

a generally white, non-cellulosic substrate for receiving the sample;

a source of Schiff's Reagent; and

a portable reflectance spectrophotometer capable of determining and reporting at least one defined color characteristic selected from hue angle, chroma or saturation, and lightness, from stained samples on said substrate.

14. A kit according to claim 8 wherein the substrate is glass fibre.

15. A process according to claim 1, wherein said specimen of biological material is the skin surface of a patient and said analyte is skin cholesterol.

16. A process of determining skin cholesterol levels of a patient, comprising:

applying to the patient's skin surface a reagent which selectively binds to skin cholesterol;

causing a color developing chemical reaction with the skin cholesterol-boundary reagent so formed, to form a color complex; and

subjecting the color complex so formed to spectrophotometric analysis to read therefrom a pre-defined characteristic of the colored complex selected from hue angle, chroma, saturation and lightness.

17. A process according to claim 16, wherein said reagent which selectively binds to skin cholesterol is selected from the group consisting of

(i) steroid glycosides, containing as an aglycone a cyclopentaneperhydrophenanthrene fragment of the furostanole or spirostanole series, and an oligosaccharide fragment including 3 to 10 monosaccharide residues with linear or branched structures,

(ii) triterpene glycosides, containing an aglycone of alpha or beta-amyryl, lupane, hopane, dommarane, linostane or holostane series, and oligosaccharides comprising saccharide residues of branched or linear structure,

(iii) hydrophobic proteins capable of discriminately forming a complex compound with cholesterol,

(iv) protein toxins, capable of discriminately forming complex compounds with cholesterol,

(v) polyens antibiotics, capable of discriminately forming complex compounds with cholesterol, and

(vi) high affinity enzymes, whose substrate is cholesterol, and which have a high affinity to it,

and formation of said colored complex is brought about by treatment of said binding agent on the skin surface first with a visualizing agent and then with an indicating agent.

18. A process according to claim 17, wherein formation of said color complex is brought about by treatment of said cholesterol binding agent on the skin surface successively with a bridging agent, a visualizing agent and an indicating agent.

19. A process according to claim 17 or claim 18, wherein said cholesterol binding agent is digitonin.

20. A process according to any one of claims 17 to 19, wherein said visualizing agent is peroxidase enzyme and said indicator agent contains hydrogen peroxide, N,N-diethel-p-phenylidene sulfate, together with appropriate stabilizers.

21. A kit for determination of skin cholesterol levels of a human patient, comprising:

a source of detecting agent, capable of binding to skin cholesterol of the patient to form a bound combination therewith on the skin;

a source of visualizing agent, capable of binding with the detecting agent-binding agent bound combination to form an optically altered complex;

a source of developing agent and means for applying the developing agent to the optically altered complex, to develop color therein; and

means for confining and for presenting said optically altered complex to a portable reflects spectrophotometer to determine therefrom a defined color characteristic selected from hue angle, chroma or saturation.

22. A kit according to claim 21, wherein said means for confining and presenting the optically altered complex to the spectrophotometer comprises a container in the form of a skin-adherent strip having at least one well passing therethrough for containment of the reagents in said well in contact against the skin of the patient.

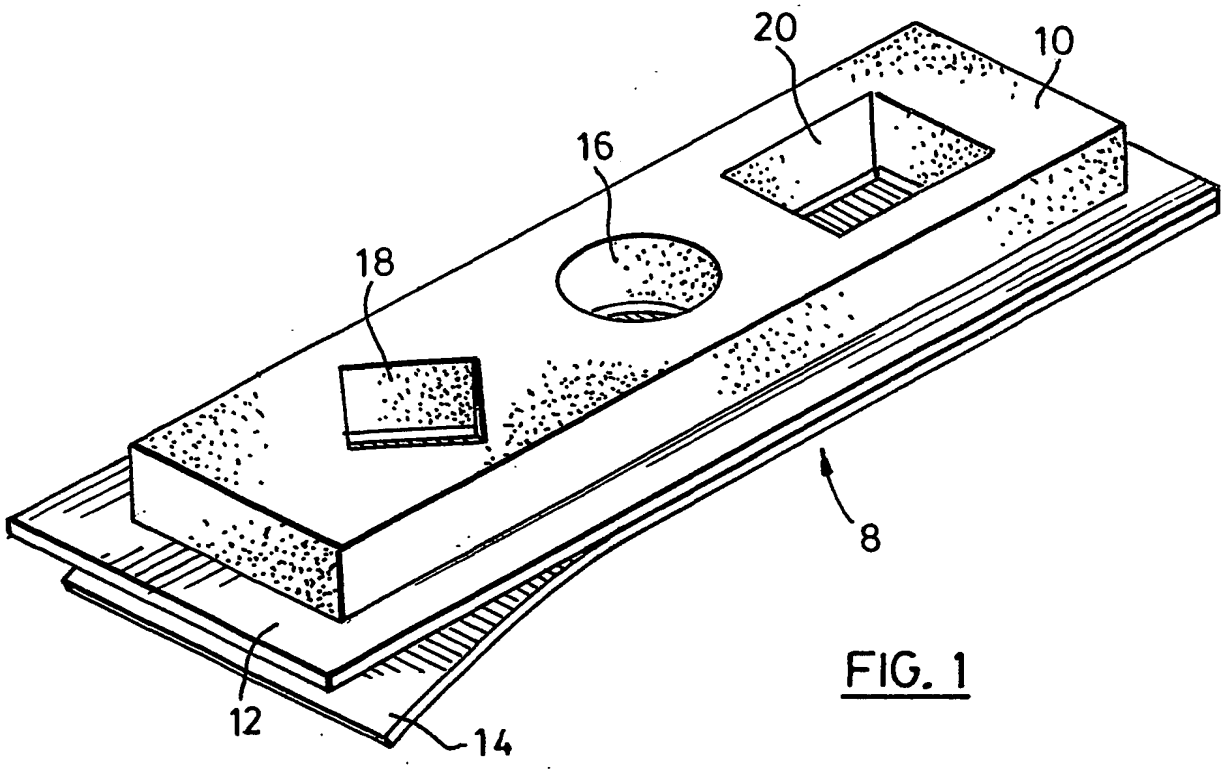

FIG. 1

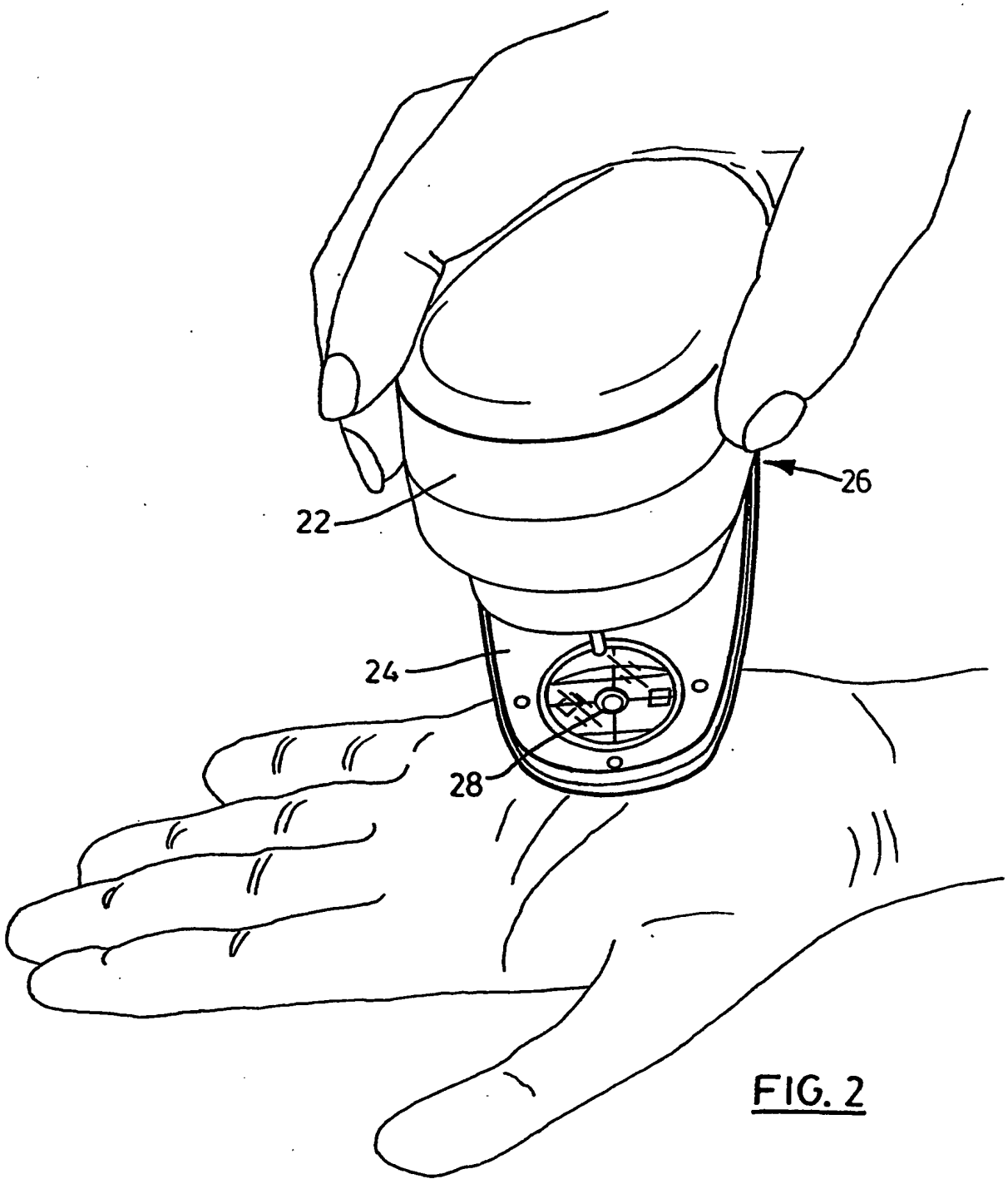

FIG. 2

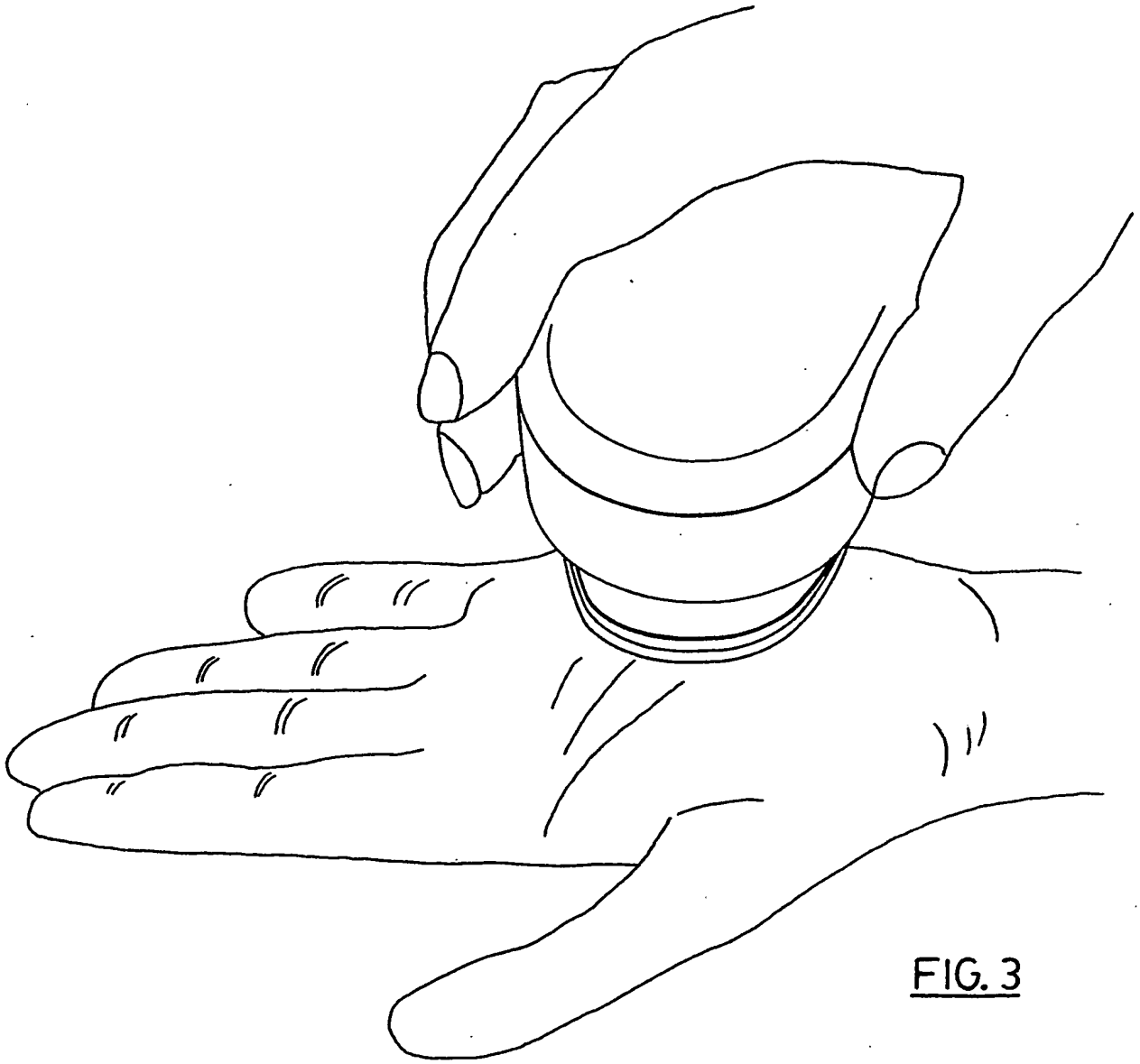

FIG. 3

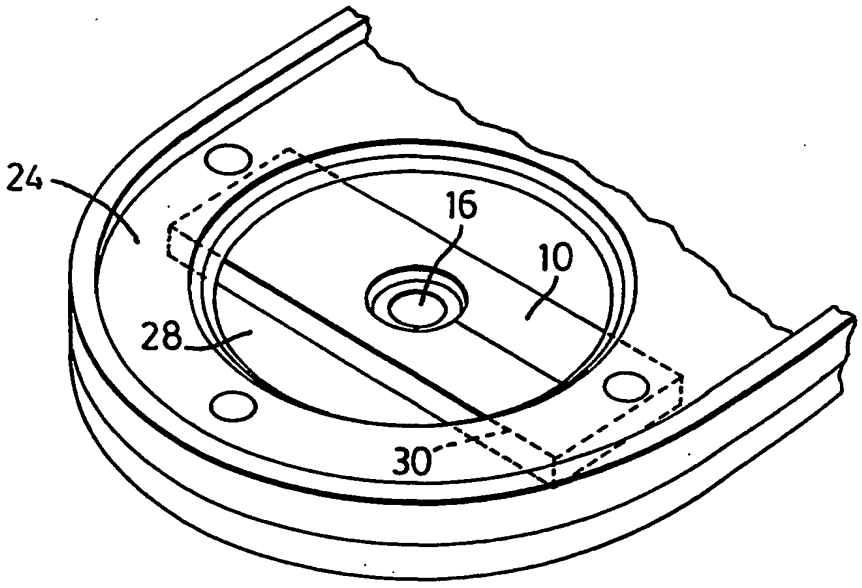

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 00251 A (BIO METRIC SYSTEMS INC) 11 January 1990 (1990-01-11)	1,7,13
A	page 15, line 32 -page 17, line 9	1,5,12
X	EP 0 893 690 A (UNIVERSITEIT GENT LAB VOOR BRO) 27 January 1999 (1999-01-27)	1,7
A	page 9, line 5 -page 10, line 6	1-6,8,10
X	WO 96 40924 A (CALGENE INC ;MCBRIDE KEVIN (US); PEAR JULIE R (US); PEREZ GRAU LUI) 19 December 1996 (1996-12-19) page 23, line 25 -page 25, line 17 page 45, line 24 -page 46, line 16	1,7
X	WO 90 11526 A (ENZYMATIX LTD) 4 October 1990 (1990-10-04)	1,2,7
A	page 7, line 15 -page 8, line 27 page 2, line 20 - line 31	1,4,5

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2001	28. 02. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Tuynman, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 14927 A (KREPINSKY JIRI J ;CHOCIEJ JACEK (CA); KANDEL GABOR P (CA); YEUNG K) 1 June 1995 (1995-06-01)	12-14
A	page 21, line 1 -page 22, line 26; claims 1-10 page 10, line 3 - line 8	1,2,4-6, 8-11
Y	WO 96 08710 A (X RITE INC) 21 March 1996 (1996-03-21)	12-14
A	figures 1,2,5 page 5, line 18 - line 23	1-11
X	GOTO M ET AL: "Chromaticity analysis of Immunostained Tumor Specimens" PATHOLOGY RESEARCH AND PRACTICE, vol. 188, no. 4,5, June 1992 (1992-06), pages 433-437, XP000972430	1,2,7,8, 11
A	abstract page 436; figure 5; table 1	1,4-6
A	EP 0 110 173 A (LIFESCAN INC) 13 June 1984 (1984-06-13) page 7, line 21 -page 8, line 15	1,6, 8-10, 12-14
A	SINCOCK ANDREW: "Computerised laser analysis of breast sections and cervical smears by transnuclear scanning." MEDICAL SCIENCE RESEARCH, vol. 24, no. 3, 1996, pages 165-166, XP000972530 ISSN: 0269-8951 the whole document	1-3,5,6, 12,13
A	GALBRAITH W ET AL: "COLORIMETRY FOR THE STAIN TECHNOLOGIST 4. ANALYSIS OF THE COMPONENTS OF COLOR DIFFERENCE" STAIN TECHNOLOGY, vol. 60, no. 4, 1985, pages 239-246, XP000972409 ISSN: 0038-9153 abstract page 242; table 3	1-3

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 37424 A (LOPUKHIN JURY MIKHAILOVICH ;PARFENOV ALEXANDR SERGEEVICH (RU)) 27 August 1998 (1998-08-27) abstract page 4, line 11 - line 20 page 6, line 23 -page 7, line 7 page 9; example 3 page 11; claims 1,3 figures 1-3	1,15-18, 21,22
A	-& EP 0 987 553 A 22 March 2000 (2000-03-22)	
A	EVELEGH M J ET AL: "Use of skin cholesterol to monitor response to cholesterol-lowering therapy." CLINICAL CHEMISTRY, vol. 45, no. 6 PART 2, June 1999 (1999-06), page A29 XP002160743 51st Annual Meeting of the American Association of Clinical Chemistry;New Orleans, Louisiana, USA; July 25-29, 1999 ISSN: 0009-9147 the whole document	15,16, 18,19
A	US 5 587 295 A (LOPUKHIN JURY M ET AL) 24 December 1996 (1996-12-24) cited in the application column 16 -column 18; example 12 column 19 -column 20; claims 1-8	16-22
A	DE 43 31 010 A (JENOPTIK JENA GMBH) 16 March 1995 (1995-03-16) abstract column 5 -column 6; claims 1-5,7,8	16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00918

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Pr test

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fe s.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,7 (partially) 2-6,8-14 (fully)

   Smear test method to analyze liquid and semi-solid body secretions collected from a patient to be diagnosed for evidence of abnormalities such as cancer.

2. Claims: 1,7 (partially) 15-22 (fully)

   Method for analyzing a patient's skin surface for cholesterol.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00918

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9000251 A	11-01-1990	NONE	
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		BR 9807594 A	22-02-2000
		EP 0987553 A	22-03-2000
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		CA 1335968 A	20-06-1995
		CN 1048925 A	30-01-1991
		CS 8900348 A	14-08-1990
		CS 8905039 A	14-08-1990
		DD 289604 A	02-05-1991
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		EP 0338189 A	25-10-1989
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		HU 56973 A	28-10-1991
		JP 1289498 A	21-11-1989
		PL 277263 A	04-09-1989
DE 4331010 A	16-03-1995	NONE	

09/828708

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/11359 A3

(51) International Patent Classification[7]: G01N 33/574

(21) International Application Number: PCT/CA00/00918

(22) International Filing Date: 4 August 2000 (04.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

2,279,793	6 August 1999 (06.08.1999)	CA
2,296,163	17 January 2000 (17.01.2000)	CA
2,306,315	20 April 2000 (20.04.2000)	CA

(71) Applicant *(for all designated States except US)*: **IMI INTERNATIONAL MEDICAL INNOVATIONS INC.** [CA/CA]; 4211 Yonge Street, Suite 300, Toronto, Ontario M2P 2A9 (CA).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **EVELEGH, Michael, J.** [CA/CA]; 5 Parkway Place, Dundas, Ontario L9H 6K3 (CA).

(74) Agents: **MITCHELL, Randall, S.** et al.; Ridout & Maybee, Suite 2400, One Queen Street East, Toronto, Ontario M5C 3B1 (CA).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
6 December 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/11359 A3

(54) Title: COLOR SPACE ANALYSIS IN BIOCHEMICAL AND IMMUNOLOGICAL ASSAYS

(57) Abstract: A process is provided for analyzing a specimen of biological material in any of a number of biochemical or immunological tests for an analyte which involves subjecting the specimen to treatment which develops a color correlating to the amount of analyte in the specimen. According to the invention at least one defined color characteristic selected from hue angle, chroma, saturation and lightness of the developed color is measured and the results of that measurement analyzed to determine the presence or concentration of the analyte in the specimen. Particular applications are to the detection of cancerous or pre-cancerous abnormalities from the analysis of lung mucus, throat mucus, cervical mucus or seminal fluid.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 00251 A (BIO METRIC SYSTEMS INC) 11 January 1990 (1990-01-11)	1,7,13
A	page 15, line 32 -page 17, line 9	1,5,12
X	EP 0 893 690 A (UNIVERSITEIT GENT LAB VOOR BRO) 27 January 1999 (1999-01-27)	1,7
A	page 9, line 5 -page 10, line 6	1-6,8,10
X	WO 96 40924 A (CALGENE INC ;MCBRIDE KEVIN (US); PEAR JULIE R (US); PEREZ GRAU LUI) 19 December 1996 (1996-12-19) page 23, line 25 -page 25, line 17 page 45, line 24 -page 46, line 16	1,7
X	WO 90 11526 A (ENZYMATIX LTD) 4 October 1990 (1990-10-04)	1,2,7
A	page 7, line 15 -page 8, line 27 page 2, line 20 - line 31	1,4,5

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2001	28. 02. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tuynman, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 14927 A (KREPINSKY JIRI J ;CHOCIEJ JACEK (CA); KANDEL GABOR P (CA); YEUNG K) 1 June 1995 (1995-06-01)	12-14
A	page 21, line 1 -page 22, line 26; claims 1-10 page 10, line 3 - line 8	1,2,4-6, 8-11
Y	WO 96 08710 A (X RITE INC) 21 March 1996 (1996-03-21)	12-14
A	figures 1,2,5 page 5, line 18 - line 23	1-11
X	GOTO M ET AL: "Chromaticity analysis of Immunostained Tumor Specimens" PATHOLOGY RESEARCH AND PRACTICE, vol. 188, no. 4,5, June 1992 (1992-06), pages 433-437, XP000972430	1,2,7,8, 11
A	abstract page 436; figure 5; table 1	1,4-6
A	EP 0 110 173 A (LIFESCAN INC) 13 June 1984 (1984-06-13) page 7, line 21 -page 8, line 15	1,6, 8-10, 12-14
A	SINCOCK ANDREW: "Computerised laser analysis of breast sections and cervical smears by transnuclear scanning." MEDICAL SCIENCE RESEARCH, vol. 24, no. 3, 1996, pages 165-166, XP000972530 ISSN: 0269-8951 the whole document	1-3,5,6, 12,13
A	GALBRAITH W ET AL: "COLORIMETRY FOR THE STAIN TECHNOLOGIST 4. ANALYSIS OF THE COMPONENTS OF COLOR DIFFERENCE" STAIN TECHNOLOGY, vol. 60, no. 4, 1985, pages 239-246, XP000972409 ISSN: 0038-9153 abstract page 242; table 3	1-3

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8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00918

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 37424 A (LOPUKHIN JURY MIKHAILOVICH ;PARFENOV ALEXANDR SERGEEVICH (RU)) 27 August 1998 (1998-08-27) abstract page 4, line 11 - line 20 page 6, line 23 -page 7, line 7 page 9; example 3 page 11; claims 1,3 figures 1-3	1,15-18, 21,22
A	-& EP 0 987 553 A 22 March 2000 (2000-03-22)	
A	EVELEGH M J ET AL: "Use of skin cholesterol to monitor response to cholesterol-lowering therapy." CLINICAL CHEMISTRY, vol. 45, no. 6 PART 2, June 1999 (1999-06), page A29 XP002160743 51st Annual Meeting of the American Association of Clinical Chemistry;New Orleans, Louisiana, USA; July 25-29, 1999 ISSN: 0009-9147 the whole document	15,16, 18,19
A	US 5 587 295 A (LOPUKHIN JURY M ET AL) 24 December 1996 (1996-12-24) cited in the application column 16 -column 18; example 12 column 19 -column 20; claims 1-8	16-22
A	DE 43 31 010 A (JENOPTIK JENA GMBH) 16 March 1995 (1995-03-16) abstract column 5 -column 6; claims 1-5,7,8	16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00918

Box I Observations where certain claims w re found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,7 (partially) 2-6,8-14 (fully)

   Smear test method to analyze liquid and semi-solid body secretions collected from a patient to be diagnosed for evidence of abnormalities such as cancer.

2. Claims: 1,7 (partially) 15-22 (fully)

   Method for analyzing a patient's skin surface for cholesterol.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00918

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9000251 A	11-01-1990	NONE	
EP 0893690 A	27-01-1999	EP 0892271 A	20-01-1999
WO 9640924 A	19-12-1996	AU 6269196 A	30-12-1996
		CA 2221747 A	19-12-1996
		EP 0835311 A	15-04-1998
		JP 11507233 T	29-06-1999
WO 9011526 A	04-10-1990	NONE	
WO 9514927 A	01-06-1995	AU 687939 B	05-03-1998
		AU 1060095 A	13-06-1995
		CA 2176508 A	01-06-1995
		EP 0731914 A	18-09-1996
		JP 9505405 T	27-05-1997
		US 5416025 A	16-05-1995
		ZA 9409290 A	21-08-1995
WO 9608710 A	21-03-1996	CA 2199868 A	21-03-1996
		CA 2199870 A	21-03-1996
		EP 0781401 A	02-07-1997
		EP 0781404 A	02-07-1997
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